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## PHYTOCHEMICALS AND PHARMACOLOGICAL SCREENING OF AQUEOUS EXTRACT OF *CASSIA FISTULA* FLOWERS

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### ABSTRACT

In the present study, the ability of scavenging free radicals of aqueous extract of *Cassia fistula* flowers was determined by using Phytochemical screening and 1, 1-diphenyl-2-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), hydrogen peroxides scavenging assay (H<sub>2</sub>O<sub>2</sub>) and superoxide anion radical scavenging (SOD). The results showed that the aqueous extract of *Cassia fistula* flowers has a significant antioxidant activity. Thus, the study suggests that *Cassia fistula* has a better source of natural antioxidants, which might be helpful in preventing the progress of oxidative stress.

### KEYWORDS

Free radical scavenging, Antioxidant, *Cassia fistula* and Oxidative stress.

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### INTRODUCTION

Herbal Medicine is defined as a branch of science in which plant based formulations are used to alleviate diseases. It is also known as botanical medicine or phytomedicine. With the advent of allopathic system of medicine, herbal medicine gradually lost its popularity among people, which is based on the fast therapeutic actions of synthetic drugs<sup>1</sup>. *Cassia* species are of medicinal interest in phytochemical and biological research due to their important medicinal values<sup>2</sup>. *Cassia fistula* Linn is a tree from Leguminosae family. It is well known as the golden shower and it is cultivated in India, Srilanka Brazil, and South Africa<sup>3</sup>. *Cassia fistula* tree has beautiful yellow flowers. The fruit is July – September

cylindrical pod and the seeds are black, sweet pulp separated by transverse partitions. The long pods are green, when unripe, turn black on ripening. Pulp is dark brown, sticky, sweet and mucilaginous, and odour characteristic<sup>4</sup>. The plant is well known with several medicinal uses, and its role in healthcare. Its main use is as a mild laxative<sup>5</sup> and it was used to treat rheumatism<sup>6,7</sup>. The plant parts were used as a therapeutic agent in the hypercholesterolaemia treatment partially because it contained a mucilage content<sup>8</sup>. *Cassia fistula* is widely grown as an ornamental plant in tropical and subtropical areas. It blooms in late spring. The golden shower tree is a medium-sized tree, growing to 10-20cm tall with fast growth. The leaves are deciduous, 15-60cm in long, and pinnate with three to eight pairs of leaflets, each leaflet 7-21 cm in long and 4-9cm in broad. The flowers are produced in pendulous racemes 20-40cm in long, each flower 4-7cm in diameter with five yellow petals of equal size and shape. The fruit is a legume, 30-60cm in long and 1.5-2.5cm (0.6-1.0 in) broad, with a pungent odour and containing several seeds. The tree has strong and very durable wood (Figure No.1). Phytochemistry of the plant reveals that it is rich with phenolic derivatives, where *Cassia fistula* leaves showed that it contained mainly flavonoids, tannins, and anthraquinones, while the fruits contained anthraquinones, flavonoids, and waxes<sup>9</sup>. It showed antipyretic and analgesic activities<sup>10</sup>. Some reports showed its antibacterial effect against a number of bacteria strains<sup>11</sup>, Antitumor<sup>12</sup>, hepatoprotective<sup>13</sup>, antifertility<sup>14</sup>, and antioxidant activities<sup>15</sup>. Moreover, their pharmacological property, the plant extract is also used as a pest and disease control agents in India<sup>16</sup>. Thus *Cassia fistula* is well known for its traditional uses and it has now found widespread in all over the world. In the present study phytochemical screening of aqueous extract of *Cassia fistula* flowers.

## MATERIAL AND METHODS

### Collection of plant material

Plant *Cassia fistula* flowers was collected from Ottapalam, Palakkad, Kerala, India in the month of

April 2018. The plant sample was thoroughly washed in the running tap water to remove the adhering dust particles and dried under the shades for about two weeks. It was made into fine powder and stored in a airtight container, which can be used for the further investigations. A voucher specimen has been deposited in the laboratory for future reference (BSI/SRC/5/23/2018/Tech.1997).

### Preparation of plant extract of *Cassia fistula* flowers

Extraction of dried powder of *Cassia fistula* flowers was carried out by boiling 20g of dried sample in boiling distilled water for 20 minutes. Then it kept for a few minutes to cool. The extraction was done by filtering using Whatman No.1 filter paper. The extracted sample was taken for the further experiments (Figure No.2).

### Preliminary phytochemicals test

Phytochemical analysis was performed to determine the presence of different phytochemicals as Described by Sadasivam and Manickam<sup>17</sup>.

### Estimation of flavonoid content

Total flavonoid content was determined according to the method<sup>18</sup>. A 1ml of each extract was mixed with 0.1ml of 10% aluminium chloride and 0.1ml of 1M potassium acetate. Methanol (2.8ml) was added and kept at room temperature for 30 min. The absorbance of the reaction mixture was measured at 415nm. The flavonoid content was expressed in mg/g, and Rutin was used as a standard compound.

### Estimation of phenol content

The total phenol content was measured using the Folin-Ciocalteu method<sup>19</sup>. Extract (100ml) was mixed with 2ml of 2% Na<sub>2</sub>CO<sub>3</sub> and allowed to stand for 2 min at room temperature. Then, 100 ml of 50% Folin-Ciocalteu phenol reagent was added. After incubation for 30 min at room temperature in darkness, the absorbance was read at 720nm. The total phenol content of the samples was expressed as mg Gallic acid per gram.

## IN VITRO RADICAL SCAVENGING ASSAY

Flowers of *Cassia fistula* were washed well with water, air dried for 8-10 days in the absence of sunlight and then powdered. Then extraction is

made using the distilled water. DPPH radical scavenging assay by the Blois<sup>20</sup>. FARP scavenging assay was estimated by the Benzie and Strain<sup>21</sup>. The hydrogen peroxide scavenging assay was estimated by the Ruch *et al*<sup>22</sup>. SOD was assayed according to the method of Kakkar *et al*<sup>23</sup>.

#### Statistical analysis

All the assays were carried out in triplicate. Experimental results are expressed as mean  $\pm$  standard deviation. The results were analyzed using one-way analysis of variance and the group means were compared using Duncan's multiple range tests using SPSS version 16.

### RESULTS AND DISCUSSION

Plants contain different phytochemicals present in different plant part and the medicinal importance due to the presence of this phytochemicals. *Cassia fistula* contains many important phytochemicals. In the present study based on the phytochemical analysis of aqueous extracts of *Cassia fistula*. Table No.1 shows the plant extracts showed the positive results, which indicates the presence of tested phytochemicals and some of the extracts show negative results. From the qualitative phytochemical test it was revealed that the aqueous extract shows the maximum presence of phytochemicals.

Flavonoids are the largest group of plant phenols and also the most studied one<sup>24</sup>. They are polyphenolic compounds that are ubiquitous in nature and occur as a glycosides, glucosides and methylated derivatives. More than 4,000 flavonoids have been recognized, many of which occur in vegetables, fruits and beverages like tea, coffee and fruit drinks<sup>25</sup>. Phenolic compounds represent the largest category of phytochemicals and are most widely distributed in the plant kingdom<sup>26</sup>. Phenolic are hydroxyl group (OH) containing class of chemical compounds where the (OH) group is bonded directly to an aromatic hydrocarbon group. Phenol (C<sub>6</sub>H<sub>5</sub>OH) is considered the simplest class of this group of natural compounds. Being a secondary metabolite, they have an important role as defense compounds. Phenolics exhibit several

properties beneficial to humans and its antioxidant properties are important in determining their role as protecting agents against free radical-mediated disease processes. The three most important groups of dietary phenolics are flavonoids, phenolicacids and polyphenols. Table No.2, shows the content of flavonoids was expressed in terms of quercetin equivalent. The total phenolic contents in the examined plant extracts using the Folin-Ciocalteu's reagent is expressed in terms of gallic acid equivalent. The values obtained for the concentration of total phenols are expressed as mg of GA/g of extract.

DPPH method measures the radical-scavenging capacity of antioxidants toward DPPH radical in organic systems and has been used extensively as a pre-screening method for new antioxidants from natural resources due to its stability, simplicity, rapidity and reproducibility. The DPPH radical in fact may be neutralized by either direct reduction via single electron transfer or by radical quenching via hydrogen atom transfer. Upon reduction, the color of the solution fades from purple to yellow and the reaction progress is conveniently monitored by a spectrophotometer<sup>27,28</sup>. The effect of flower extracts and standard on DPPH radical was compared and shown in Figure. The scavenging effect increases with the concentration of standard and samples. At 1000 $\mu$ g/mL concentration, *Cassia fistula* possessed 40.298% scavenging activity on DPPH and IC<sub>50</sub> of the extract was 1539.615 $\pm$ 646.895. The potential increase in the % of inhibition is due to the scavenging property of aqueous extract of *Cassia fistula*.

Antioxidants with reducing power are those that can act as electron donors and can reduce the oxidised intermediates of lipid peroxidation processes, allowing them to act as primary and secondary antioxidants<sup>29</sup>. Such as antioxidants react with potassium ferricyanide (Fe<sup>3+</sup>) to form potassium ferrocyanide (Fe<sup>2+</sup>), which then reacts with ferric chloride (Fe<sup>3+</sup>) to form ferrous complexes (Fe<sup>2+</sup>) that have a maximum absorbance at 700nm<sup>30</sup>. Percentage scavenging activities of hydroxyl radical examined at different concentrations of *Cassia*

*fistula* were revealed in Figure No.5. *Cassia fistula* were exhibited a maximum hydroxyl radical scavenging activity of 9.742(%) at 1000µg/ml whereas ascorbic acid was found to be (7.593%) at 1000µg/ml. The IC<sub>50</sub> of the extract was 2886.87± 2197.57ml.

H<sub>2</sub>O<sub>2</sub> is a non radical compound, and is of potential biological significance because of its ability to penetrate biological membranes. H<sub>2</sub>O<sub>2</sub> itself is not very reactive, but it can sometimes be toxic to the cell because it may give rise to hydroxyl radical in the cells (single oxygen and HO radicals. Thus, removal of H<sub>2</sub>O<sub>2</sub> is very essential to protect the biological system in general, and food components, in particular. It was reported that extracts of some brown seaweeds registered more than (90%) H<sub>2</sub>O<sub>2</sub> scavenging activity<sup>31</sup>, thereby supporting the very fact that brown seaweeds are rich source of natural antioxidant compounds, which can scavenge H<sub>2</sub>O<sub>2</sub> radical. Many other species of seaweeds were also reported in literature to possess potential H<sub>2</sub>O<sub>2</sub> scavenging activity<sup>32</sup>.

Percentage scavenging activity of hydroxyl radical examined at different concentrations of *Cassia fistula* was revealed in Figure. *Cassia fistula* were exhibited a maximum hydroxyl radical scavenging activity of (73.098%) at 250µg/ml and ascorbic acid (standard) was found to be (75.922%) at 250µg/ml. The IC<sub>50</sub> of the extract was 190.028 ± 81.351ml.

Superoxide is a highly reactive molecule that reacts with various substances produced through metabolic processes. Percentage scavenging activity of superoxide anion examined at different concentrations of *Cassia fistula* aqueous extract was revealed in Table No.3. *Cassia fistula* were exhibited a maximum activity of superoxide dismutase and was found to be 0.26±0.055. Superoxide anion radical is one of the strongest reactive oxygen species among the free radicals that are generated after oxygen is taken into living cells. Superoxide anion changes to other harmful ROS and free radicals such as hydrogen peroxide and hydroxyl radical, which induce oxidative damage<sup>33</sup>.

**Table No.1: Phytochemical evaluation of aqueous extract of *Cassia fistula* flowers**

S.No	Phytochemical	Aqueous extract
1	Phenols	+
2	Flavanoids	+
3	Alkaloids	+
4	Tannins	+
5	Saponins	+
6	Carbohydrate	+
7	Glycosides	-
8	Proteins	++
9	Amino acid	++

(+) Presence (++) High levels of phytochemicals (-) Absence

**Table No.2: Quantitative analysis of *Cassia fistula* flowers in water**

S.No	Phytochemicals	Aqueous extract
1	Flavanoids	0.074mg/g
2	Phenols	.5mg/g

**Table No.3: Levels of enzymatic antioxidants presents in the water flower extract of *Cassia fistula***

S.No	Parameters	Values
1	Superoxide Dismutase	0.26±0.055

Values are expressed as Mean±SD (n=3)



Figure No.1: *Cassia fistula* flower



Figure No.2: *Cassia fistula* shade dry sample

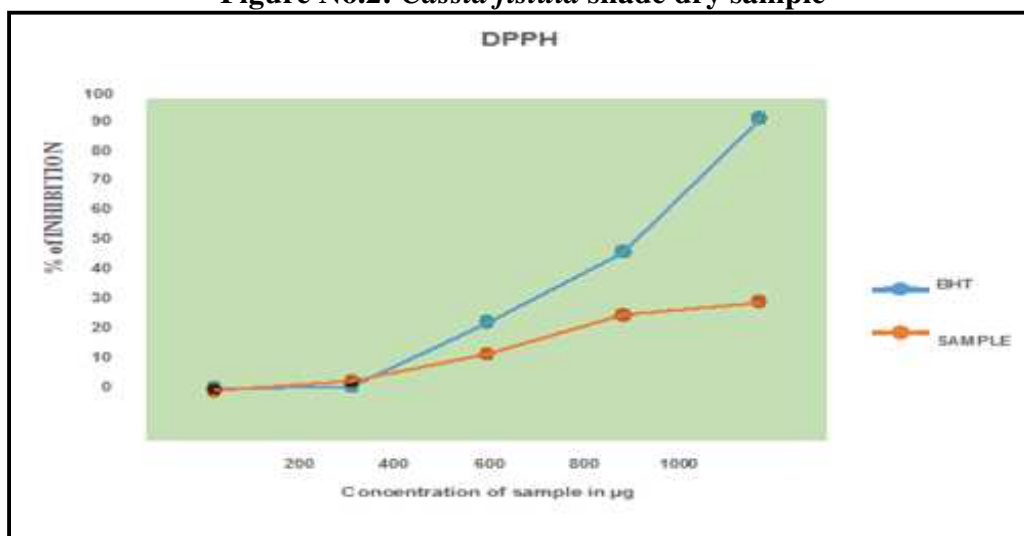


Figure No.3: DPPH of *Cassia fistula*

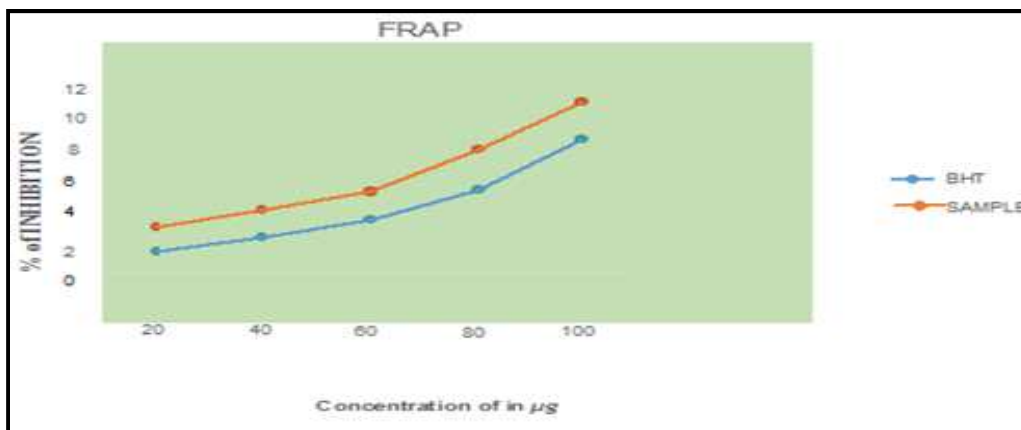


Figure No.4: Ferric chloride reducing power of *Cassia fistula*

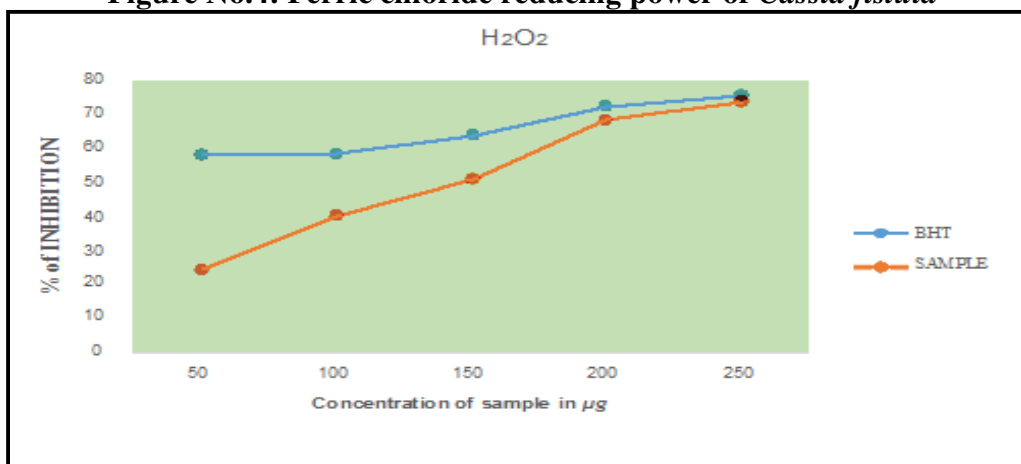


Figure No.5: Hydrogen Peroxide Radical scavenging ability of *Cassia fistula*

## CONCLUSION

This study suggests that the *Cassia fistula* aqueous extract of possess high free radical scavenging activity which might be useful for further studies to unravel novel treatment strategies for diseases associated with free radical induced tissue damage.

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## CONFLICT OF INTEREST

The authors declare that they don't have any conflict of interest.

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